Reproductive biology of *Grewia occidentalis* L. (Tiliaceae)

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Grewia occidentalis flowers are monomorphic, protandrous and dichogamous, the latter being complete. The flowers are self-incompatible and the pollen–ovule ratio of 8664:1 is indicative of a xenogamous species with relatively inefficient pollen transfer. *G. occidentalis* is pollinated by *Apis mellifera* and two species of *Xylocopa*. Pollinator availability appears not to have an adverse effect on seed set. The behavioural pattern of most of the honey-bees on the flowers is such that pollination does not take place during the majority of visits.

Die blomme van *Grewia occidentalis* is monomorfies, digogaam en protandries. Digogamie is volledig. Die blomme is selfonverenigbaar en die verhouding van stuifmeelkorrels tot saadknoppe van 8664:1 dui op 'n kruisbestuifde spesie waarvan stuifmeeloordraging relatief oneffektief is. *G. occidentalis* word bestuif deur *Apis mellifera* en twee *Xylocopa* spesies. Dit wil voorkom asof die beskikbaarheid van bestuiwers geen negatiewe invloed op saadset het nie. Die gedragspatroon van die meeste heuningbye tydens besoeke aan die blomme is van so 'n aard dat bestuiwing nie kan plaasvind nie.

Keywords: Dichogamy, *Grewia occidentalis*, Tiliaceae, mellitophily, self-incompatibility.

Introduction

Grewia occidentalis L. (voucher: *P.C. & L. Zietsman 597*, NMB), occurs commonly in the Bloemfontein area, especially in the more densely vegetated areas associated with drainage lines and the south-facing slopes of koppies. It flowers between October and the end of May (Zietsman *et al.* 1989). The flowers of *G. occidentalis* show some, but not all, of the characteristics of typical bee-pollinated flowers (Faegri & van der Pijl 1979). The purple flowers are mechanically strong with adequate facilities for insect landing and offer a moderate supply of nectar hidden behind the nectariferous claw. As flowers in general often attract a variety of pollinator groups despite floral specialization (Baker 1961, 1963), *G. occidentalis* may not be exclusively mellitophillic. The aim of this study was to investigate the breeding system and pollination mechanism of *G. occidentalis*.

Material and Methods

Study area

Pollination experiments and observations were conducted at Bloemfontein Botanical Garden (29 26 AA) which is just north of the city and is considered to be part of the Dry *Cymbopogon–Themeda* veld (Acocks 1988). Phenological data was collected at Glen Agricultural College (28 26 CD). At both localities, *G. occidentalis* occurs naturally in undisturbed veld. Observations showed that the two populations flower concurrently. As the two sites are furthermore very similar in species composition, data collected from both localities were used in this study.

Breeding system

Pollen–ovule ratio

Pollen grains from each of five anthers, collected from five different flowers on different individual trees just before the start of anthesis, were separately suspended in 10 ml of a 0.1% (m/v) aniline blue staining solution. Counts were made of 0.5 ml of each of these suspensions using a Zeiss stereomicroscope. The mean value of three replicates was used to determine the number of pollen grains per anther. This figure was multiplied by the mean number of stamens per flower, as was determined by counting the number of stamens on 10 different flowers collected from five different trees. The number of ovules per flower was determined for 10 flowers from as many trees.

Pollen viability

Pollen viability was determined by examining 10 flowers from 10 different trees using Alexander's viability stain (Alexander 1969) and by means of the fluorochromatic (FCR) test procedure (Heslop-Harrison *et al.* 1984).

Pollination experiments

Hand pollination experiments were conducted using two different methods. In the first method, 10 flowers from five different trees were pollinated and left on the trees. From these, 25 flowers were collected after 24 h and fixed in FAA. After staining with decolorized aniline blue, pollen germination and pollen tube growth in pistils of these flowers were observed with a Zeiss Axioskop fluorescent microscope. The remaining 25 flowers were left intact to test for open seed set. In the second method the flowers were picked and immediately put on a 5% (m/v) agar medium. These latter flowers were pollinated in the laboratory after which they were kept in closed plastic containers at room temperature, fixed in FAA after 24 h and prepared for microscope observations as described. Pollination treatments involved the following:

(a) Open pollination: 25 flowers at each study area were marked and checked regularly to determine natural rates of pollination and fruit set. Initiation of fruit development was visible within three weeks after anthesis. Evaluation of fruit set was based on counts made after two months.

(b) Autogamy: flowers were bagged and left unmanipulated to test for self-pollination.
(c) Self-compatibility: flowers were self-pollinated and bagged.
(d) Xenogamy: flowers were crossed with pollen collected from trees not closer than 10 m from the tree used for the experiment.
(e) Apomixis: flowers were emasculated and bagged.
(f) Geitonogamy: flowers were pollinated with pollen collected from different flowers on the same tree and bagged.

Pollination mechanism

Floral behaviour

Floral behaviour of *G. occidentalis* was monitored in 10 different individuals at the study area. Anthesis and movement of the sepals, petals and stamens as well as the development of the pistil were monitored at 15 min intervals from 06:00 to 20:00 on five different days. The beginning of anthesis in these individuals was monitored during the same period.

Rewards and attractants

Pollen. To obtain an indication of the dissemination of pollen grains during the first eight hours following anthesis, five anthers of four different individual flowers were used for pollen counts. Pollen grains were counted as described for the determination of the pollen–ovule ratio.

Nectar. The relative quantity of nectar (standing crop) was evaluated on a subjective scale of 0 – 3, on three different days for each of five randomly picked flowers (unbagged) in the different stages of anthesis (a value of 0 indicates no nectar production whereas a value of 3 represents a high production). A mean value was determined for each stage.

Osmophores. To detect odoriferous substances, flowers representing the different phases of floral behaviour were kept in closed vials for three hours after which accumulated odoriferous substances, if present, could be detected by smell on opening of the vial (Van Wyk & Lowrey 1988). For the location of osmophores fresh flowers were placed for 5 h in a 0.1% (m/v) solution of neutral red (Vogel 1962). Flowers were handled carefully because any bruised tissue is stained by neutral red. This can lead to incorrect interpretation of results.

Pollen vectors: wind and insects

Vaseline-coated microscope slides were randomly placed in five different trees. The slides were removed after 24 h, stained with aniline blue and examined for pollen. Insects visiting the flowers were collected in killing jars, killed in 100% ethyl acetate, and scrutinized for the presence and position of *G. occidentalis* pollen on their bodies.

Behavioural observations of flower visitors were made for a total of three days during December 1989 from 06:00 until 18:00. Every hour, 5 min were spent at each of five randomly chosen trees where flower visitors were observed. Insect visitation rates were recorded and expressed as number of insects per hour per 30 cm of flower-bearing twig. The manner in which the pollinators made contact with the rewards offered, as well as the duration of visits, were also noted. On three different occasions observations were made between 18:00 and 21:00 to determine if the flowers receive nocturnal visitors.

Results

Breeding System

Pollen ovule ratio and pollen viability

The average number of grains per flower was 34657 ± 917 while ovule number was constant at four per flower. The P/O ratio was 8664:1. Staining with Alexander's stain indicated that 95% of the pollen grains were viable. The FCR test procedure indicated that only 69% of the pollen with live protoplast were germinable, thus indicating that only about 65% of the pollen grains were viable.

Pollination experiments

(a) Open pollination: Fruits were formed in 34% of the flowers marked and left exposed to pollinators. Of these, 45% produced the maximum of four seeds per fruit (Figure 1). In 28% of marked flowers, one or two seeds were produced. Natural seed set is therefore 12%.
(b) Autogamy: None of the 25 flowers that were bagged and left unpollinated produced any seeds.
(c) Self-compatibility: None of the self-pollinated flowers set seed. In only one of the flowers (n = 25) that were self-pollinated *in vivo* and fixed in FAA were germinated pollen grains and pollen tube growth in the style noted when observed with a fluorescence microscope after staining.
(d) Xenogamy: The majority of pollen grains on cross-pollinated styles germinated and the pollen tubes penetrated the length of the style. In 38% of the flowers that were left intact, fruits were formed. Actual seed set in these fruits was 15% which does not differ significantly from the figure obtained for natural seed set.
(e) Apomixis and geitonogamy: None of the flowers emasculated and bagged to test for apomixis, or cross-pollinated within trees to test for geitonogamy, set seed. In the latter case, observation under UV light, after staining with decolorized aniline blue, failed to reveal pollen tubes in the style.

Pollination mechanisms

Floral behaviour

An individual flower lasts for two days and during this period floral behaviour can be divided into three different phases (Figures 2a – 2c).

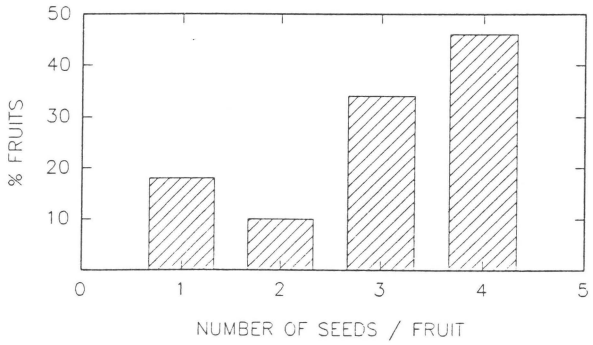

Figure 1 Seed set in *Grewia occidentalis* (n = 50).

(a) Anthesis: The timing of anthesis differs between individual flowers on the same tree. Anthesis in one group of flowers starts at approximately 08:00 – 9:00, in another at 12:00 – 13:00 and in the last group at approximately 15:00 – 16:00. The duration of anthesis differs between individual flowers but takes between 1 and 2 h. It starts when slits appear between the sepals (Figure 2a) and ends when the sepals and petals are completely unfolded and perpendicular to the flower base. Anthers dehisce in the bud before anthesis.

(b) Male phase: At the start of the male phase the pistil is already fully developed and the stigma protrudes just above the anthers. This phase can be subdivided into two shorter stages:
   (i) The stamens, which up to this stage were folded tightly against the pistil, start to unfold (Figure 2b). The stamens bend as much as 45° away from the pistil. Loosely packed, slightly sticky pollen is available to pollinators during this stage. After approximately 5 h, 60% of the pollen grains have been dispersed with the remaining grains packed on the inside of the anthers where they are probably unavailable to pollinators. Small quantities of nectar are produced during this stage in the nectariferous claw which is located at the base of each petal.
   (ii) This stage, during which the stamens fold back against the pistil (Figure 2c), marks the end of the male phase and starts approximately 8 – 10 h after anthesis.

(c) Female phase: The female phase lasts for approximately 18 h. Only very slight morphological changes are apparent on the stigma during this phase. The stigma papillae become more pronounced and there are indications of stigmatic exudate. The rate of nectar production appears to be higher during the female phase than during either of the male stages, resulting in drops of nectar at the base of each petal. At this stage the remainder of the pollen grains are inaccessible to pollinators. This phase ends when the petals fold back to their original position as before anthesis, covering the pistil (Figure 2d).

Pollinator rewards and attractants

Most of the pollen grains are dispersed during the male phase in the first two hours following anthesis (Figure 3). During this period the number of available pollen grains decreases by 34%, and continues to drop steadily until only 10% are dispersed during the last 2-h interval. Nectar is available just after anthesis and its relative quantity starts to increase during the later male stage (b)(ii) and is at its highest during the female stage. The flowers had no detectable scent and staining with neutral red did not reveal any osmophores.

Pollen vectors

Pollen grains were found on 4% of the slides used in this experiment. Honey-bees (*Apis mellifera*) and two carpenter bee species (*Xylocopa*; Apidae) were the only insects visiting *G. occidentalis* which carried abundant pollen of this species. Pollen is deposited on the undersides of the bodies and on the heads of pollinators during visits to flowers in the male phase. Carpenter bees visited the flowers on only three occasions during the period of observation and behavioural data was therefore collected only for honey-bees. The only other tree species in the study area that was visited by honey-bees was *Olea europaea* subsp. *africana*.

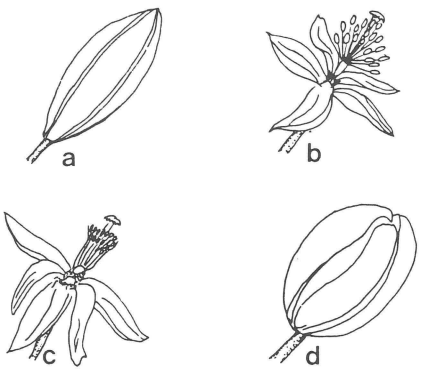

Figure 2 Schematic representation of four stages of floral development in *Grewia occidentalis*. (a) Bud stage; (b) male stage; (c) female stage; and (d) end of female stage.

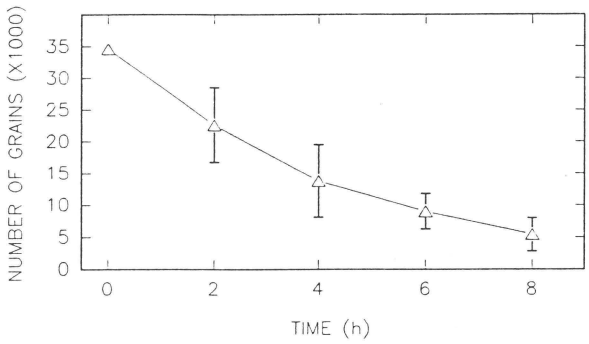

Figure 3 Rate of pollen dispersion in *Grewia occidentalis* following the first nine hours after anthesis.

Pollinator behaviour

Each 30 cm of flower-bearing twig received an average of 86 pollinators (honey-bees) per hour during the observation period of 10 h. No nocturnal pollinators were observed. Three different pollinator visitation behaviours were distinguished. Some pollinators land on top of the flower on the anthers and circle around, collecting pollen (18%). Another group, which comprised 64% of the visits, collected only nectar and after landing on the petals, gained access to this reward, which is hidden behind the nectariferous claw, through a space between the bases of adjoining petals. A third group (18% of all visits) collected both rewards by first landing on the anthers and collecting pollen and then by hanging upside down from their position on the anthers to feed on the nectar. There was no significant difference in the length of floral visits for bees collecting pollen and/or nectar.

Discussion

The temporal separation of the reproductive organs of *G. occidentalis* flowers suggests that the monomorphic flowers of this species are dichogamous. Although only very slight morphological changes on the stigma are apparent during the female phase, it occurs after most, if not all, accessible

pollen grains have been dispersed, indicating protandry. In this species the temporal separation of pollen and receptive stigma is presented in a single flower and it can be regarded as intrafloral dichogamy. Furthermore, dichogamy is complete, as is indicated by the lack of overlap between pollen presentation and stigma receptivity (Lloyd & Webb 1986). The timing of anthesis in the flowers on an individual tree differs and results in three synchronized groups. This is regarded as multi-cycled dichogamy (Lloyd & Webb 1986). The flowers of *G. occidentalis* differ in this respect from other representatives of the Tiliaceae which have hermaphroditic flowers, as is the case in species of *Leuhea* (Haber & Frankie 1982). The pollen–ovule ratio of *G. occidentalis* is indicative of a xenogamous species and implies that pollen transfer in this species is relatively inefficient (Cruden 1977).

Results obtained from experimental pollinations conducted in the laboratory and in the field, indicate that *G. occidentalis* is self-incompatible and does not reproduce by apomixis. This species appears to have a sporophytic SI system as pollen germination of self-pollen is inhibited on the stigmatic surface. In cross-pollinated styles pollen grains germinated and sent tubes down the length of the style.

The floral phases in laboratory-conducted pollinations were delayed compared with the flowers pollinated and left intact on the trees. In the styles of 4% of the flowers that were self-pollinated in the laboratory, germinated pollen grains and pollen tube growth were noted. This is acceptable within the limits set for self-incompatibility by Bawa (1974). This was, however, not observed in styles of flowers that were self-pollinated by hand and left intact on the trees. The high humidity in the closed containers in which the flowers were kept (Carter & McNeilly 1975; Palloix *et al.* 1985), as well as the delayed floral phases, probably contributed to overcoming the SI barriers in these flowers. This experimental procedure should therefore be used with utmost care to avoid making incorrect conclusions.

As pollen grains were found on only 4% of the glass slides used when testing for wind pollination, it is unlikely that wind contributes towards pollination of *G. occidentalis*. Although the experimental lay-out tested only for within-tree movement of pollen, thus actually testing for possible geitonogamous crosses, much less pollen movement would be expected between trees. Since *G. occidentalis* is self-incompatible it can be assumed that wind plays no role in pollination of this species. Although some of the characteristics that Faegri and van der Pijl (1979) include in their syndrome of honey-bee blossoms, apply to *G. occidentalis* flowers, pollination in *G. occidentalis* appears not to be specialized.

The behaviour of pollinators (honey-bees) can be divided into one of three categories: nectar collection, pollen collection, or pollen and nectar collection. Nectar collection alone does not result in stigmatic contact, but when combined with pollen collection, pollen deposition on stigmas does occur. Pollen does not appear to be the main attractant for the pollinators. Pollen-collecting visitors constitute no more than 36% of all visits as compared with 64% of visits exclusively for nectar collection. This may be a result of the limited supply of pollen on an individual flower. The behavioural pattern of *G. occidentalis* pollinators appears to be unspecialized and inefficient. Since honey-bees are particularly abundant in the study areas, and have been observed visiting the flowers at any time of the day, seed set in *G. occidentalis* does not appear to be pollinator-limited. This is supported by the actual figure for seed set in experimental cross-pollinations of 15% as compared with 12% in the control.

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